Chlorophyll concentration in water samples

DETERMINATION BY SPECTROPHOTOMETER

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1 Introduction

On many occasions we refer to algae as any flora that can grow in water. Most often these are simply aquatic plants, the removal of which is very complex due to the seeds and roots that allow them to sprout again and again. Algae, however, lack stem, leaves, or vascular tissue. The determination of chlorophyll is a parameter that can indicate the quality of water in terms of the concentration of phytoplankton, which are microscopic algae. With this parameter it is possible to know the standard of living that a water reserve can hold, in relation to the fish and plants that live in it.

There are generally three chlorophylls in phytoplankton: chlorophyll a, b and c. Chlorophyll is in the order of 1 to 2% of the dry weight of plankton-type algae. It is a complex molecule that has a magnesium atom in the center with a ring of porphyrins, which would be the head of the molecule, and a tail of CH_2-CH_3.

Chlorophyll can be easily detected by its response to light through optical measurement from discrete water samples. This allows us to carry out periodic analysis of water quality and generate a schedule of this evolution.

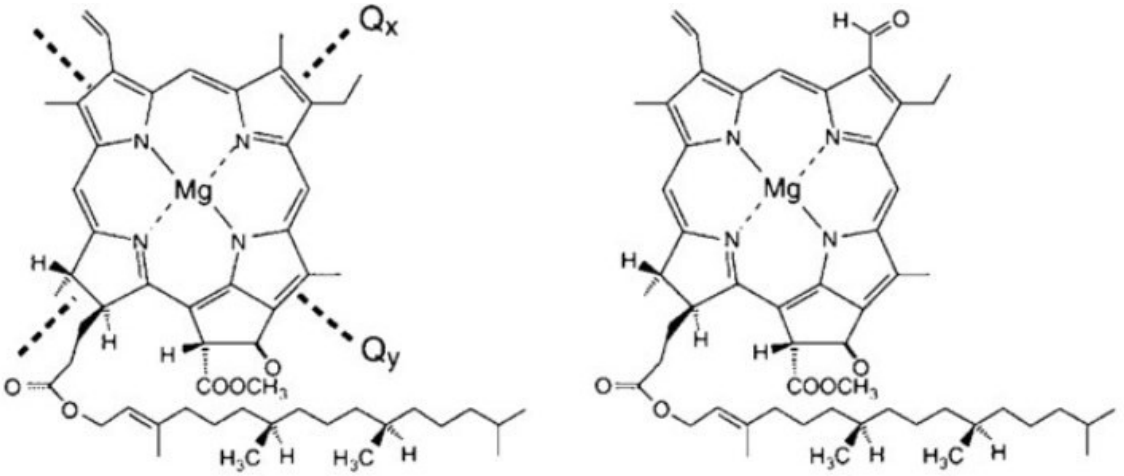

Illustration 1- Representation of chlorophyll molecules a and b.

2 Procedure

1. A sample of water is collected, trying to make it a non-stagnant area so that the mixture is homogeneous. It is placed in one liter polyethylene bottle, clean and sterile.
2. Filter 250 mL of water using GF/F quality fiberglass filters, Whatman type.
3. With the sample filtered, the filter should be taken with great caution not to contaminate the sample. Using tweezers, it is bent and placed in a test tube coved with a thread.
4. Add 5 mL of acetone at 90% concentration, close the tube with the filter and acetone and cover it with aluminum foil to preserve it from light.
5. Shake for 1 minute and store the tube in the refrigerator for 24 hours and in complete darkness, without opening it.
6. After this period, a pipette removes the acetone from the tube and placed in a clean tube.

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7. Centrifuge the acetone from the new tube for 10 minutes at 3000 rpm.
8. Remove between 1 and 3 mL from the tube and place them in the spectrophotometer cell.
9. Choose the size of the cell that provides an absorbance greater than 0.2 and less than1, at 664nm, being usual between 1 and 2 cm.
10. Measure sample absorbances at 664 nm, 647 nm and 630 nm on the spectrophotometer.

3 Calculation

Based on Jeffrey & Humphrey's (1975) equations, the concentrations are calculated as follows:

$$C_a = (11.85 \cdot A664) - (1.54 \cdot A647) - (0.08 \cdot A630)$$

$$C_b = (-5.47 \cdot A664) - (21.03 \cdot A647) - (2.66 \cdot A630)$$

$$C_c = (-1.67 \cdot A664) - (7.6 \cdot A647) - (24.52 \cdot A630)$$

It should be noted that the reading at 750nm is used as a turbidity correction, so this reading will have to be subtracted from the absorbance values of the other wavelengths, which are the corrected absorbances, as reflected in the equations.
The values should be substituted in the following concentration equations:

Chlorophyll a (mg/L) = [Ca · v] / [L·V]

Chlorophyll b (mg/L) = [Cb · v] / [L·V]

Chlorophyll c (mg/L) = [Cc · v] / [L·V]

Where,
v: volume of acetone used to extract pigments (mL).
L: length of the spectrophotometer cell (cm).
V: volume of filtered water (L).

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